NCBI Protein

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM Bo

Search Protein for FGFR3 Go Clear

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Entrez Protein

Field: **Protein Name**

Display Summary Show: 20 Send to Text

Items 1-8 of 8 One page.

Related resources

1: AAF97749 BLink, Links
mutant fibroblast growth factor receptor 3; FGFR3 [Homo sapiens]
gi|9719332|gb|AAF97749.1|AF238374_1[9719332]

2: BAA84726 BLink, Domains, Links
FGFR3/4 [Lethenteron reissneri]
gi|6002415|dbj|BAA84726.1|[6002415]

3: BAA84725 BLink, Links
FGFR3/4b [Eptatretus burgeri]
gi|6002413|dbj|BAA84725.1|[6002413]

4: BAA84724 BLink, Links
FGFR3/4a [Eptatretus burgeri]
gi|6002411|dbj|BAA84724.1|[6002411]

5: AAB35669 BLink, Links
fibroblast growth factor receptor 3, FGFR3 {Ala391Glu, transmembrane domain} [human, Crouzon syndrome and acanthosis nigricans patient, Peptide Partial Mutant, 24 aa]
gi|1245905|gb|AAB35669.1||bbm|381440|bbs|175068[1245905]

6: AAB35668 BLink, Links
fibroblast growth factor receptor 3, FGFR3 {Gly380Arg, transmembrane domain} [human, achondroplasia patient, Peptide Partial Mutant, 24 aa]
gi|1245904|gb|AAB35668.1||bbm|381439|bbs|175067[1245904]

7: AAB35667 BLink, Links
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gi|1245903|gb|AAB35667.1||bbm|381438|bbs|175066[1245903]

8: AAB33323 BLink, Links
fibroblast growth factor receptor 3; FGFR3 [Homo sapiens]
gi|914202|gb|AAB33323.1||bbm|365610|bbs|163272[914202]

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Items 1-8 of 8 One page.

Revised: July 5, 2002.

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i686-pc-linux-gnu Jan 21 2003 17:57:06

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☐ 1: AAA58470. growth factor rec...[gi:182565]

BLink, Domains, Links

```
LOCUS       AAA58470                731 aa            linear   PRI 31-DEC-1994
DEFINITION  growth factor receptor.
ACCESSION   AAA58470
VERSION     AAA58470.1  GI:182565
DBSOURCE    locus HUMFGFLR accession M64347.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 731)
  AUTHORS   Thompson,L.M., Plummer,S., Schalling,M., Altherr,M.R.,
            Gusella,J.F., Housman,D.E. and Wasmuth,J.J.
  TITLE     A gene encoding a fibroblast growth factor receptor isolated from
            the Huntington disease gene region of human chromosome 4
  JOURNAL   Genomics 11 (4), 1133-1142 (1991)
  MEDLINE   92147110
   PUBMED   1664411
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                     /db_xref="taxon:9606"
                     /map="4p16.3 D4599"
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      121 rgehriggik lrhqqwslvm esvvpsdrgn ytcvvenkfg sirqtytldv lersphrpil
      181 qaglpanqta vlgsdvefhc kvysdaqphi qwlkhvevng skvgpdgtpy vtvlktagan
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      301 ilsygvgffl filvvaavtv crlrsppkkg lgsptvhkis rfplkrqvsl esnasmssnt
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      541 rdlaarnvlv tednvmkiad fglardvhnl dyykkttngr lpvkwmapea lfdrvythqs
      601 dvwsfgvllw eiftlggspy pgipveelfk llkeghrmdk pancthdlym imrecwhaap
      661 sqrptfkqlv edldrvltvt stdeyldlsa pfeqyspggq dtpssssgd dsvfahdllp
      721 pappssggsr t
//
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Revised: July 5, 2002.

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Jan 21 2003 18:08:12

NCBI formatting BLAST

Nucleotide Protein Translations Retrieve results for an RID

Your request has been successfully submitted and put into the Blast Queue.

Query = (731 letters)

Putative conserved domains have been detected, click on the image below for detailed results.

The request ID is 1043363757-023776-3483

Format! or Reset all

The results are estimated to be ready in 29 seconds but may be done sooner.

Please press "FORMAT!" when you wish to check your results. You may change the formatting options for your result via the form below and press "FORMAT!" again. You may also request results of a different search by entering any other valid request ID to see other recent jobs.

Format

Show ☑ Graphical Overview ☑ Linkout ☑ Sequence Retrieval ☑ NCBI-gi Alignment in HTML

Number of: Descriptions 100 Alignments 50

Alignment view Pairwise

Format for PSI-BLAST ☐ with inclusion threshold: 0.005

Limit results by entrez query or select from: (none)

Expect value range:

gi|15281418|gb|AAK94209.1| keratinocyte growth factor receptor 2 isoform K-sam-IIC2 [Homo sapiens]
Length = 785

Score = 1003 bits (2593), Expect = 0.0
Identities = 495/705 (70%), Positives = 564/705 (80%), Gaps = 11/705 (1%)

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Sbjct: 74  KDGVHLGPNNRTVLIGEYLQIKGATPRDSGLYACTASRTVDSETWYFMVNVTDAISSGDD 133

Query: 57  -XXXXXXXXXXXXXXXXXXAPYWTRPERMDKKLLAVPAANTVRFRCPAAGNPTPSISWLK 115
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Sbjct: 194 NGKEFKQEHRIGGYKVRNQHWSLIMESVVPSDKGNYTCVVENEYGSINHTYHLDVVERSP 253

Query: 176 HRPILQAGLPANQTAVLGSDVEFHCKVYSDAQPHIQWLKHVEVNGSKVGPDGTPYVTVLK 235
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Query: 471 TQGGPLYVLVEYAAKGNLREFLRARRPPGLDYSFDTCKPPEEQLTFKDLVSCAYQVARGM 530
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Query: 195 DVEFHCKVYSDAQPHIQWLKHVEVNGSKVGPDGTPYVTVLKTAGANTTDKELEVLSLHNV 254
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Query: 371 -GEGPTLANVSELELPADPKWELSRARLTLGKPLGEGCFGQVVMAEAIGIDKDRAAKPVT 429
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AAH04257. ret proto-oncogen...[gi:13279041]

BLink, Links

LOCUS AAH04257 1072 aa linear PRI 12-JUL-2001
DEFINITION ret proto-oncogene (multiple endocrine neoplasia MEN2A, MEN2B and medullary thyroid carcinoma 1, Hirschsprung disease) [Homo sapiens].
ACCESSION AAH04257
VERSION AAH04257.1 GI:13279041
DBSOURCE locus BC004257 accession BC004257.1
KEYWORDS .
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (residues 1 to 1072)
AUTHORS Strausberg,R.
TITLE Direct Submission
JOURNAL Submitted (01-MAR-2001) National Institutes of Health, Mammalian Gene Collection (MGC), Cancer Genomics Office, National Cancer Institute, 31 Center Drive, Room 11A03, Bethesda, MD 20892-2590, USA
REMARK NIH-MGC Project URL: http://mgc.nci.nih.gov
COMMENT Contact: MGC help desk
Email: cgapbs-r@mail.nih.gov
Tissue Procurement: ATCC
cDNA Library Preparation: Rubin Laboratory
cDNA Library Arrayed by: The I.M.A.G.E. Consortium (LLNL)
DNA Sequencing by: Genome Sequence Centre,
BC Cancer Agency, Vancouver, BC, Canada
info@bcgsc.bc.ca
Steven Jones, Jennifer Asano, Ian Bosdet, Yaron Butterfield, Susanna Chan, Readman Chiu, Chris Fjell, Erin Garland, Ran Guin, Letticia Hsiao, Martin Krzywinski, Reta Kutsche, Oliver Lee, Soo Sen Lee, Victor Ling, Carrie Mathewson, Candice McLeavy, Steven Ness, Pawan Pandoh, Anna-Liisa Prabhu, Parvaneh Saeedi, Jacqueline Schein, Duane Smailus, Michael Smith, Lorraine Spence, Jeff Stott, Michael Thorne, Miranada Tsai, Natasja van den Bosch, Jill Vardy, George Yang, Scott Zuyderduyn, Marco Marra.

Clone distribution: MGC clone distribution information can be found through the I.M.A.G.E. Consortium/LLNL at: http://image.llnl.gov
Series: IRAL Plate: 13 Row: e Column: 3
This clone was selected for full length sequencing because it passed the following selection criteria: Hexamer frequency ORF analysis.
Method: conceptual translation.
FEATURES Location/Qualifiers

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      241 avctvhagar eevvmvpfpv tvydeddsap tfpagvdtas avvefkrked tvvatlrvfd
      301 advvpasgel vrrytstllp gdtwaqqtfr vehwpnetsv qangsfvrat vhdyrlvlnr
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      541 cewrqgdgkg itrnfstcsp stktcpdghc dvvetqdini cpqdclrgsi vgghepgepr
      601 gikagygtcn cfpeeekcfc epediqdplc delcrtviaa avlfsfivsv llsafcihcy
      661 hkfahkppis saemtfrrpa qafpvsysss sarrpsldsm enqvsvdafk iledpkwefp
      721 rknlvlgktl gegefgkvvk atafhlkgra gyttvavkml kenaspselr dllsefnvlk
      781 qvnhphvikl ygacsqdgpl lliveyakyg slrgflresr kvgpgylgsg gsrnsssldh
      841 pderaltmgd lisfawqisq gmqylaemkl vhrdlaarni lvaegrkmki sdfglsrdvy
      901 eedsyvkrsq gripvkwmai eslfdhiytt qsdvwsfgvl lweivtlggn pypgipperl
      961 fnllktghrm erpdncseem yclmlqcwkq epdkrpvfad iskdlekmmv krrdyldlaa
     1021 stpsdsliyd dglseeetpl vdcnnaplpr alpstwienk lygrishaft rf
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WEST

End of Result Set

Generate Collection Print

L2: Entry 1 of 1 File: USPT Oct 16, 2001

DOCUMENT-IDENTIFIER: US 6303301 B1
TITLE: Expression monitoring for gene function identification

US PATENT NO. (1):
6303301

Detailed Description Text (10):
(B) Hybridizing nucleic acids to high density array

Detailed Description Text (21):
Background: The terms "background" or "background signal intensity" refer to hybridization signals resulting from non-specific binding, or other interactions, between the labeled target nucleic acids and components of the oligonucleotide array (e.g. the oligonucleotide probes, control probes, the array substrate, etc.). Background signals may also be produced by intrinsic fluorescence of the array components themselves. A single background signal can be calculated for the entire array, or a different background signal may be calculated for each target nucleic acid. In a preferred embodiment, background is calculated as the average hybridization signal intensity for the lowest 5% to 10% of the probes in the array, or, where a different background signal is calculated for each target gene, for the lowest 5% to 10% of the probes for each gene. Of course, one of skill in the art will appreciate that where the probes to a particular gene hybridize well and thus appear to be specifically binding to a target sequence, they should not be used in a background signal calculation. Alternatively, background may be calculated as the average hybridization signal intensity produced by hybridization to probes that are not complementary to any sequence found in the sample (e.g. probes directed to nucleic acids of the opposite sense or to genes not found in the sample such as bacterial genes where the sample is mammalian nucleic acids). Background can also be calculated as the average signal intensity produced by regions of the array that lack any probes at all.

Detailed Description Text (27):
Mismatch control: The term "mismatch control" or "mismatch probe" refer to a probe whose sequence is deliberately selected not to be perfectly complementary to a particular target sequence. For each mismatch (MM) control in a high-density array there typically exists a corresponding perfect match (PM) probe that is perfectly complementary to the same particular target sequence. The mismatch may comprise one or more bases. While the mismatch(s) may be located anywhere in the mismatch probe, terminal mismatches are less desirable as a terminal mismatch is less likely to prevent hybridization of the target sequence. In a particularly preferred embodiment, the mismatch is located at or near the center of the probe such that the mismatch is most likely to destabilize the duplex with the target sequence under the test hybridization conditions.

Detailed Description Text (43):
This invention provides methods, compositions and apparatus for interrogating the genetic network and for studying normal and abnormal functions for specific genes. The methods involve quantifying the level of expression of a large number of genes. In some preferred embodiments, a high density oligonucleotide array is used to hybridize with a target nucleic acid sample to detect the expression level of a large number of genes, preferably more than 10, more preferably more than 100, and most preferably more than 1000 genes.

Detailed Description Text (46):
In general, genetic regulatory relationships can be explored to detect potential

mutations once a target gene's down-stream regulated genes are identified. In one embodiment of the invention, the expression of several down-stream positively regulated genes is monitored using a high density oligonucleotide array. Diminished expression of those positively regulated genes indicates a possible malfunction of the target gene. Such malfunction may indicate the presence of a potential mutation in the target gene. Other mutation detection methods, such as the tiling methods, can then be used to confirm and to detect the nature of the mutation. Many sets of such down-stream positively regulated genes, each set of genes being regulated by a target gene, can be monitored simultaneously. This simultaneous detection of mutations in many genes is an major improvement over prior art methods. It will be apparent to those skilled in the art that negatively regulated down-stream genes can also be used in a similar manner.

Detailed Description Text (52):
High density arrays are particularly useful for monitoring the expression control at the transcriptional, RNA processing and degradation level. The fabrication and application of high density arrays in gene expression monitoring have been disclosed previously in, for example, WO 97/10365, WO 92/10588, U.S. application Ser. No. 08/772,376 filed Dec. 23, 1996; Ser. No. 08/529,115 filed on Sep. 15, 1995; Ser. No. 08/168,904filed Dec. 15, 1993; Ser. No. 07/624,114 filed on Dec. 6, 1990, Ser. No. 07/362,901 filed Jun. 7, 1990, all incorporated herein for all purposed by reference. In some embodiment using high density arrays, high density oligonucleotide arrays are synthesized using methods such as the Very Large Scale Immobilized Polymer Synthesis (VLSIPS) disclosed in U.S. Pat. No. 5,445,934 incorporated herein for all purposes by reference. Each oligonucleotide occupies a known location on a substrate. A nucleic acid target sample is hybridized with a high density array of oligonucleotides and then the amount of target nucleic acids hybridized to each probe in the array is quantified. One preferred quantifying method is to use confocal microscope and fluorescent labels. The GeneChip.RTM. system (Affymetrix, Santa Clara, Calif.) is particularly suitable for quantifying the hybridization; however, it will be apparent to those of skill in the art that any similar systems or other effectively equivalent detection methods can also be used.

Detailed Description Text (53):
High density arrays are suitable for quantifying a small variations in expression levels of a gene in the presence of a large population of heterogeneous nucleic acids. Such high density arrays can be fabricated either by de novo synthesis on a substrate or by spotting or transporting nucleic acid sequences onto specific locations of substrate. Nucleic acids are purified and/or isolated from biological materials, such as a bacterial plasmid containing a cloned segment of sequence of interest. Suitable nucleic acids are also produced by amplification of templates. As a nonlimiting illustration, polymerase chain reaction, and/or in vitro transcription, are suitable nucleic acid amplification methods.

Detailed Description Text (54):
Synthesized oligonucleotide arrays are particularly preferred for this invention. Oligonucleotide arrays have numerous advantages, as opposed to other methods, such as efficiency of production, reduced intra- and inter array variability, increased information content and high signal-to-noise ratio.

Detailed Description Text (55):
Preferred high density arrays for gene function identification and genetic network mapping comprise greater than about 100, preferably greater than about 1000, more preferably greater than about 16,000 and most preferably greater than 65,000 or 250,000 or even greater than about 1,000,000 different oligonucleotide probes, preferably in less than 1 cm.sup.2 of surface area. The oligonucleotide probes range from about 5 to about 50 or about 500 nucleotides, more preferably from about 10 to about 40 nucleotide and most preferably from about 15 to about 40 nucleotides in length.

Detailed Description Text (59):
Transcriptional factors are proteins that recognize and bind cis-acting trancriptional elements. Often, but not always, those factors contain two domains: a DNA-binding domain and an activation domain. The DNA-binding domain typically contains the leucine zipper motif the helix-loop-helix motif, helix-turn-helix motif and/or the zinc finger motif. Transcriptional factors are encoded by their own genes. Therefore, the expression level of transcriptional factors may affect the expression of other genes. Those trans-acting factors are integral part of the genetic network. In some embodiments of the invention, the expression of transcriptional factors is monitored by the use of a high density array. In some other embodiments, the expression of transcriptional factors are monitored at the protein level by the use of two

dimensional gel electrophoresis, mass-spectrometry or immunological methods.

Detailed Description Text (60):
In some preferred embodiments, direct measurement, such as the nuclear run-on assay, of transcriptional rate is employed. In such embodiments, nuclei are isolated from cells of interest. Isolated nuclei are incubated with labeled nucleotides for a period of time. Transcripts are then hybridized with probes. In some preferred embodiments, transcripts are quantified with high density nucleic acid array.

Detailed Description Text (63):
Many genes are known to have several alternative promoters, the use of each promoter resulting one particular transcript. The use of alternative promoters is frequently employed to regulate tissue specific gene expression. For example, human dystrophin gene has at least seven promoters. The most 5' upstream promoter is used to transcribe a brain specific transcript; a promoter 100 kb down-stream from the first promoter is used to transcribe a muscle specific transcript and a promoter 100 kb downstream of the second promoter is used to transcribe Purkinje cell specific transcript. The use of alternative promoters is part of the gene network control mechanism. In several embodiments of the invention, the use of alternative promoters can be monitored and mapped to resolve its regulatory relationship among genes. In one preferred embodiment, a high density oligonucleotide array is used to monitor the use of specific promoters by measuring the amount of transcripts resulting from each of the alternative promoters. In such embodiments, probes are designed to be specific for each of the exons that are alternatively used. A high density oligonucleotide array is particularly useful for this purpose because of the flexibility of probe design. However, one of ordinary skill in the art would appreciate that other methods, such as DNA arrays, RT-PCR, differential display, optical oligonucleotide sensors, can also be used to monitor the alternative use of promoters.

Detailed Description Text (64):
Similarly, alternative splicing and polyadenylation are also important mechanisms for regulating gene activity, frequently in a tissue specific manner. In eukaryotes, nascent pre-mRNAs are generally not translated into proteins. Rather, they are processed in several ways to generate mature mRNAs. RNA splicing is the most common method of RNA processing. Nascent pre-mRNAs are cut and pasted by specialized apparatus called splicesomes. Some non coding regions transcribed from the intron regions are excised. Exons are linked to form a contiguous coding region ready for translation. In some splicing reactions, a single type of nascent pre-mRNAs are used to generate multiple types of mature RNA by a process called alternative splicing in which exons are alternatively used to form different mature mRNAs which code for different proteins. For example, the human Calcitonin gene (CALC) is spliced as calcitonin, a circulating Ca.sup.2+ homeostatic hormone, in the thyroid; and as calcitonin gene-related peptide (CGRP), a neuromodulatory and trophic factor, in the hypothalamus (See, Hodges and Bernstein, 1994, Adv. Genet., 31, 207-281). This diversity of gene product is achieved by a combination of alternative splicing and alternative adenylation. Regulation of the alternative splicing and adenylation is a part of the genetic network. In some embodiments of the invention, alternative splicing are monitored. Many methods are suitable for detecting alternative splicing and adenylation. High density oligonucleotide arrays are particularly suitable for this purpose because of their design flexibility. Oligonucleotide probes against specific sequence diversity can be readily synthesized and used to detect the level of each of the sequences produced by alternative splicing and adenylation.

Detailed Description Text (65):
RNA editing is another form of post-transcriptional processing. For example, certain genes, such as the Wilm's tumor susceptibility gene (WTI), apolipoprotein (APOB) gene, and glutamate receptor gene undergo C->U or U->C substitution editing events (See, Scott, 1995, Cell, 81, 833-836). In the liver, the human APOB gene encodes a 4536 amino acid product. In the intestine, however, the same gene encodes a 2152 amino acid product. The smaller product is due to the addition of a stop codon during RNA editing. High density oligonucleotide arrays are particularly suitable for detecting RNA editing events. Because single base mismatches can be readily identified, oligonucleotide probes against each of potential RNA editing products are fabricated on a single substrate to detect the level of those products by specific hybridization.

Detailed Description Text (71):
One preferred method for massive parallel gene expression monitoring is based upon high density nucleic acid arrays. Nucleic acid array methods for monitoring gene expression are disclosed and discussed in detail in PCT Application WO 092.10588 (published on

Jun. 25, 1992), all incorporated herein by reference for all purposes.

Detailed Description Text (72):
Generally those methods of monitoring gene expression involve (a) providing a pool of target nucleic acids comprising RNA transcript(s) of one or more target gene(s), or nucleic acids derived from the RNA transcript(s); (b) hybridizing the nucleic acid sample to a high density array of probes and (c) detecting the hybridized nucleic acids and calculating a relative and/or absolute expression (transcription, RNA processing or degradation) level.

Detailed Description Text (82):
Methods of "quantitative" amplification are well known to those of skill in the art. For example, quantitative PCR involves simultaneously co-amplifying a known quantity of a control sequence using the same primers. This provides an internal standard that may be used to calibrate the PCR reaction. The high density array may then include probes specific to the internal standard for quantification of the amplified nucleic acid.

Detailed Description Text (87):
It will be appreciated by one of skill in the art that the direct transcription method described above provides an antisense (aRNA) pool. Where antisense RNA is used as the target nucleic acid, the oligonucleotide probes provided in the array are chosen to be complementary to subsequences of the antisense nucleic acids. Conversely, where the target nucleic acid pool is a pool of sense nucleic acids, the oligonucleotide probes are selected to be complementary to subsequences of the sense nucleic acids. Finally, where the nucleic acid pool is double stranded, the probes may be of either sense as the target nucleic acids include both sense and antisense strands.

Detailed Description Text (89):
(B) Hybridizing nucleic acids to high density arrays

Detailed Description Text (91):
One of skill in the art will appreciate that an enormous number of array designs are suitable for the practice of this invention. The high density array will typically include a number of probes that specifically hybridize to the sequences of interest. In addition, in a preferred embodiment, the array will include one or more control probes.

Detailed Description Text (92):
The high density array chip includes "test probes." Test probes could be oligonucleotides that range from about 5 to about 45 or 5 to about 500 nucleotides, more preferably from about 10 to about 40 nucleotides and most preferably from about 15 to about 40 nucleotides in length. In other particularly preferred embodiments the probes are 20 or 25 nucleotides in length. In another preferred embodiments, test probes are double or single strand DNA sequences. DNA sequences are isolated or cloned from nature sources or amplified from nature sources using nature nucleic acid as templates. These probes have sequences complementary to particular subsequences of the genes whose expression they are designed to detect. Thus, the test probes are capable of specifically hybridizing to the target nucleic acid they are to detect.

Detailed Description Text (93):
In addition to test probes that bind the target nucleic acid(s) of interest, the high density array can contain a number of control probes. The control probes fall into three categories referred to herein as 1) normalization controls; 2) expression level controls; and 3) mismatch controls.

Detailed Description Text (94):
Normalization controls are oligonucleotide or other nucleic acid probes that are complementary to labeled reference oligonucleotides or other nucleic acid sequences that are added to the nucleic acid sample. The signals obtained from the normalization controls after hybridization provide a control for variations in hybridization conditions, label intensity, "reading" efficiency and other factors that may cause the signal of a perfect hybridization to vary between arrays. In a preferred embodiment, signals (e.g., fluorescence intensity) read from all other probes in the array are divided by the signal (e.g., fluorescence intensity) from the control probes thereby normalizing the measurements.

Detailed Description Text (95):
Virtually any probe may serve as a normalization control. However, it is recognized that hybridization efficiency varies with base composition and probe length. Preferred

normalization probes are selected to reflect the average length of the other probes present in the array, however, they can be selected to cover a range of lengths. The normalization control(s) can also be selected to reflect the (average) base composition of the other probes in the array, however in a preferred embodiment, only one or a few normalization probes are used and they are selected such that they hybridize well (i.e. no secondary structure) and do not match any target-specific probes.

Detailed Description Text (99):
The high density array may also include sample preparation/amplification control probes. These are probes that are complementary to subsequences of control genes selected because they do not normally occur in the nucleic acids of the particular biological sample being assayed. Suitable sample preparation/amplification control probes include, for example, probes to bacterial genes (e.g., Bio B) where the sample in question is a biological from a eukaryote.

Detailed Description Text (101):
In a preferred embodiment, oligonucleotide probes in the high density array are selected to bind specifically to the nucleic acid target to which they are directed with minimal non-specific binding or cross-hybridization under the particular hybridization conditions utilized. Because the high density arrays of this invention can contain in excess of 1,000,000 different probes, it is possible to provide every probe of a characteristic length that binds to a particular nucleic acid sequence. Thus, for example, the high density array can contain every possible 20-mer sequence complementary to an IL-2 mRNA.

Detailed Description Text (102):
However, there may exist 20-mer subsequences that are not unique to the IL-2 mRNA. Probes directed to these subsequences are expected to cross-hybridize with occurrences of their complementary sequence in other regions of the sample genome. Similarly, other probes simply may not hybridize effectively under the hybridization conditions (e.g., due to secondary structure, or interactions with the substrate or other probes). Thus, in a preferred embodiment, the probes that show such poor specificity or hybridization efficiency are identified and may not be included either in the high density array itself (e.g., during fabrication of the array) or in the post-hybridization data analysis.

Detailed Description Text (103):
In addition, in a preferred embodiment, expression monitoring arrays are used to identify the presence and expression (transcription) level of genes which are several hundred base pairs long. For most applications it would be useful to identify the presence, absence, or expression level of several thousand to one hundred thousand genes. Because the number of oligonucleotides per array is limited in a preferred embodiment, it is desired to include only a limited set of probes specific to each gene whose expression is to be detected.

Detailed Description Text (104):
As disclosed in U.S. application Ser. No. 08/772,376, probes as short as 15, 20, or 25 nucleotide are sufficient to hybridize to a subsequence of a gene and that, for most genes, there is a set of probes that performs well across a wide range of target nucleic acid concentrations. In a preferred embodiment, it is desirable to choose a preferred or "optimum" subset of probes for each gene before synthesizing the high density array.

Detailed Description Text (105):
2. Forming High Density Arrays.

Detailed Description Text (106):
Methods of forming high density arrays of oligonucleotides, peptides and other polymer sequences with a minimal number of synthetic steps are known. The oligonucleotide analogue array can be synthesized on a solid substrate by a variety of methods, including, but not limited to, light-directed chemical coupling, and mechanically directed coupling See Pirrung el al., U.S. Pat. No. 5,143,854 (see also PCT Application No. WO 90/15070) and Fodor et al., PCT Publication Nos. WO 92/10092 and WO 93/09668 and U.S. Ser. No. 07/980,523 which disclose methods of forming vast arrays of peptides, oligonucleotides and other molecules using, for example, light-directed synthesis techniques. See also, Fodor el al., Science, 251, 767-77 (1991). These procedures for synthesis of polymer arrays are now referred to as VLSIPS.TM. procedures. Using the VLSIPS.TM. approach, one heterogeneous array of polymers is converted, through simultaneous coupling at a number of reaction sites, into a different heterogeneous

array. See, U.S. application Ser. Nos. 07/796,243 and 07/980,523.

Detailed Description Text (107):
The development of VLSIPS.TM. technology as described in the above-noted U.S. Pat. No. 5,143,854 and PCT patent publication Nos. WO 90/15070 and 92/10092, is considered pioneering technology in the fields of combinatorial synthesis and screening of combinatorial libraries. More recently, patent application Ser. No. 08/082,937, filed Jun. 25, 1993, describes methods for making arrays of oligonucleotide probes that can be used to check or determine a partial or complete sequence of a target nucleic acid and to detect the presence of a nucleic acid containing a specific oligonucleotide sequence.

Detailed Description Text (108):
In brief, the light-directed combinatorial synthesis of oligonucleotide arrays on a glass surface proceeds using automated phosphoramidite chemistry and chip masking techniques. In one specific implementation, a glass surface is derivatized with a silane reagent containing a functional group, e.g., a hydroxyl or amine group blocked by a photolabile protecting group. Photolysis through a photolithogaphic mask is used selectively to expose functional groups which are then ready to react with incoming 5'-photoprotected nucleoside phosphoramidites. The phosphoramidites react only with those sites which are illuminated (and thus exposed by removal of the photolabile blocking group). Thus, the phosphoramidites only add to those areas selectively exposed from the preceding step. These steps are repeated until the desired array of sequences have been synthesized on the solid surface. Combinatorial synthesis of different oligonucleotide analogues at different locations on the array is determined by the pattern of illumination during synthesis and the order of addition of coupling reagents.

Detailed Description Text (111):
In addition to the foregoing, additional methods which can be used to generate an array of oligonucleotides on a single substrate are described in co-pending applications Ser. No. 07/980,523, filed Nov. 20, 1992, and 07/796,243, filed Nov. 22, 1991 and in PCT Publication No. WO 93/09668. In the methods disclosed in these applications, reagents are delivered to the substrate by either (1) flowing within a channel defined on predefined regions or (2) "spotting" on predefined regions or (3) through the use of photoresist. However, other approaches, as well as combinations of spotting and flowing, may be employed. In each instance, certain activated regions of the substrate are mechanically separated from other regions when the monomer solutions are delivered to the various reaction sites.

Detailed Description Text (113):
Thereafter, a monomer B is coupled to second selected regions, some of which may be included among the first selected regions. The second selected regions will be in fluid contact with a second flow channel(s) through translation, rotation, or replacement of the channel block on the surface of the substrate; through opening or closing a selected valve; or through deposition of a layer of chemical or photoresist. If necessary, a step is performed for activating at least the second regions. Thereafter, the monomer B is flowed through or placed in the second flow channel(s), binding monomer B at the second selected locations. In this particular example, the resulting sequences bound to the substrate at this stage of processing will be, for example, A, B, and AB. The process is repeated to form a vast array of sequences of desired length at known locations on the substrate.

Detailed Description Text (116):
High density nucleic acid arrays can be fabricated by depositing presynthezied or natural nucleic acids in predined positions. Synthesized or natural nucleic acids are deposited on specific locations of a substrate by light directed targeting and oligonucleotide directed targeting. Nucleic acids can also be directed to specific locations in much the same manner as the flow channel methods. For example, a nucleic acid A can be delivered to and coupled with a first group of reaction regions which have been appropriately activated. Thereafter, a nucleic acid B can be delivered to and reacted with a second group of activated reaction regions. Nucleic acids are deposited in selected regions. Another embodiment uses a dispenser that moves from region to region to deposit nucleic acids in specific spots. Typical dispensers include a micropipette or capillary pin to deliver nucleic acid to the substrate and a robotic system to control the position of the micropipette with respect to the substrate. In other embodiments, the dispenser includes a series of tubes, a manifold, an array of pipettes or capillary pins, or the like so that various reagents can be delivered to the reaction regions simultaneously.

Detailed Description Text (120):
In general, there is a tradeoff between hybridization specificity (stringency) and signal intensity. Thus, in a preferred embodiment, the wash is performed at the highest stringency that produces consistent results and that provides a signal intensity greater than approximately 10% of the background intensity. Thus, in a preferred embodiment, the hybridized array may be washed at successively higher stringency solutions and read between each wash. Analysis of the data sets thus produced will reveal a wash stringency above which the hybridization pattern is not appreciably altered and which provides adequate signal for the particular oligonucleotide probes of interest.

Detailed Description Text (123):
Altering the thermal stability (T.sub.m) of the duplex formed between the target and the probe using, e.g., known oligonucleotide analogues allows for optimization of duplex stability and mismatch discrimination. One useful aspect of altering the T.sub.m arises from the fact that adenine-thymine (A-T) duplexes have a lower T.sub.m than guanine-cytosine (G-C) duplexes, due in part to the fact that the A-T duplexes have 2 hydrogen bonds per base-pair, while the G-C duplexes have 3 hydrogen bonds per base pair. In heterogeneous oligonucleotide arrays in which there is a non-uniform distribution of bases, it is not generally possible to optimize hybridization for each oligonucleotide probe simultaneously. Thus, in some embodiments, it is desirable to selectively destabilize G-C duplexes and/or to increase the stability of A-T duplexes. This can be accomplished, e.g., by substituting guanine residues in the probes of an array which form G-C duplexes with hypoxanthine, or by substituting adenine residues in probes which form A-T duplexes with 2,6 diaminopurine or by using the salt tetramethyl ammonium chloride (TMACl) in place of NaCl.

Detailed Description Text (124):
Altered duplex stability conferred by using oligonucleotide analogue probes can be ascertained by following, e.g., fluorescence signal intensity of oligonucleotide analogue arrays hybridized with a target oligonucleotide over time. The data allow optimization of specific hybridization conditions at, e.g., room temperature (for simplified diagnostic applications in the future).

Detailed Description Text (134):
Means of detecting labeled target (sample) nucleic acids hybridized to the probes of the high density array are known to those of skill in the art. Thus, for example, where a colorimetric label is used, simple visualization of the label is sufficient. Where a radioactive labeled probe is used, detection of the radiation (e.g. with photographic film or a solid state detector) is sufficient.

Detailed Description Text (135):
In a preferred embodiment, however, the target nucleic acids are labeled with a fluorescent label and the localization of the label on the probe array is accomplished with fluorescent microscopy. The hybridized array is excited with a light source at the excitation wavelength of the particular fluorescent label and the resulting fluorescence at the emission wavelength is detected. In a particularly preferred embodiment, the excitation light source is a laser appropriate for the excitation of the fluorescent label.

Detailed Description Text (136):
The confocal microscope may be automated with a computer-controlled stage to automatically scan the entire high density array. Similarly, the microscope may be equipped with a phototransducer (e.g., a photomultiplier, a solid state array, a CCD camera, etc.) attached to an automated data acquisition system to automatically record the fluorescence signal produced by hybridization to each oligonucleotide probe on the array. Such automated systems are described at length in U.S. Pat. No: 5,143,854, PCT Application 20 92/10092, and copending U.S. application Ser. No. 08/195,889 filed on Feb. 10, 1994. Use of laser illumination in conjunction with automated confocal microscopy for signal detection permits detection at a resolution of better than about 100 .mu.m, more preferably better than about 50 .mu.m, and most preferably better than about 25 .mu.m.

Detailed Description Text (137):
One of skill in the art will appreciate that methods for evaluating the hybridization results vary with the nature of the specific probe nucleic acids used as well as the controls provided. In the simplest embodiment, simple quantification of the fluorescence intensity for each probe is determined. This is accomplished simply by

measuring probe signal strength at each location (representing a different probe) on the high density array (e.g., where the label is a fluorescent label, detection of the amount of florescence (intensity) produced by a fixed excitation illumination at each location on the array). Comparison of the absolute intensities of an array hybridized to nucleic acids from a "test" sample with intensities produced by a "control" sample provides a measure of the relative expression of the nucleic acids that hybridize to each of the probes.

Detailed Description Text (140):
In addition, the provision of appropriate controls permits a more detailed analysis that controls for variations in hybridization conditions, cell health, non-specific binding and the like. Thus, for example, in a preferred embodiment, the hybridization array is provided with normalization controls. These normalization controls are probes complementary to control sequences added in a known concentration to the sample. Where the overall hybridization conditions are poor, the normalization controls will show a smaller signal reflecting reduced hybridization. Conversely, where hybridization conditions are good, the normalization controls will provide a higher signal reflecting the improved hybridization. Normalization of the signal derived from other probes in the array to the normalization controls thus provides a control for variations in hybridization conditions. Typically, normalization is accomplished by dividing the measured signal from the other probes in the array by the average signal produced by the normalization controls. Normalization may also include correction for variations due to sample preparation and amplification. Such normalization may be accomplished by dividing the measured signal by the average signal from the sample preparation/amplification control probes (e.g., the Bio B probes). The resulting values may be multiplied by a constant value to scale the results.

Detailed Description Text (141):
As indicated above, the high density array can include mismatch controls. In a preferred embodiment, there is a mismatch control having a central mismatch for every probe (except the normalization controls) in the array. It is expected that after washing in stringent conditions, where a perfect match would be expected to hybridize to the probe, but not to the mismatch, the signal from the mismatch controls should only reflect non-specific binding or the presence in the sample of a nucleic acid that hybridizes with the mismatch. Where both the probe in question and its corresponding mismatch control both show high signals, or the mismatch shows a higher signal than its corresponding test probe, there is a problem with the hybridization and the signal from those probes is ignored. The difference in hybridization signal intensity between the target specific probe and its corresponding mismatch control is a measure of the discrimination of the target-specific probe. Thus, in a preferred embodiment, the signal of the mismatch probe is subtracted from the signal from its corresponding test probe to provide a measure of the signal due to specific binding of the test probe.

Detailed Description Text (145):
In addition to high density nucleic acid arrays, other methods are also useful for massive gene expression monitoring. Differential display, described by Liang, P. and Pardee, A. B. (Differential Display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257:967-971, 1992, incorporated herein by reference for all purposes) provides a useful mean for distinguishing gene expression between two samples. Serial analysis of gene expression, described by Velculescu et al. (Serial Analysis of Gene Expression. Science, 270:484-487, 1995, incorporated herein by reference for all purposes) provides another method for quantative and qualitative analysis of gene expression. Optical fiber oligonucleotide sensors, described by Ferguson et al. (A Fiber-optic DNA biosensor microarray for the analysis of gene expression. Nature-Biotechnology 14:1681-1684, 1996), can also be used for gene expression monitoring.

Detailed Description Text (177):
1. High Density Array Design and Fabrication

Detailed Description Text (179):
Approximately 65,000 unique DNA probes (10.sup.7 unique probes/50 mm.sup.2 area) were synthesized on a 1.2 cm.sup.2 glass slide. A set of 4 different oligonucleotide arrays that include more than 6,500 human gene sequences derived from the GenBank (http://www.ncbi.nlm.nih.gov) and dbEST databases were generated. These arrays were used to monitor and compare the expression of>6,500 genes in parallel from normal and malignant breast tissue cell lines.

Detailed Description Text (181):

Labeled RNA for array hybridization experiments was derived from the malignant breast cell line BT-474 and normal breast tissue from primary cell line HT-125. BT-474 was isolated from a solid, invasive ductal carcinoma of the breast and is tumorigenic in athymic nude mice. HT-125 was obtained from a cell line derived from normal breast tissue peripheral to an infiltrating ductal carcinoma. Using a p53 genotyping array (Affymetrix, Santa Clara, Calif.), the p53 gene in BT-474 was analyzed to find potential mutations. FIG. 5 shows the results of the genotypic analysis demonstrating that there was a G to A base change resulting in a E to K amino acid change at position 285 in exon 8, the p53 DNA binding domain. Methods for this genotypic analysis is disclosed in the U.S. patent application Ser. No. 08/143,312, filed on Oct. 26, 1993, WO 95/11995, U.S. Pat. No. 5,677,195, U.S. Ser. No. 08/327,525, filed Oct. 21, 1994, and WO 97/29212, incorporated herein by reference for all purposes.

Detailed Description Text (185):
The labeled cRNA was then fragmented in the presence of heat and Mg.sup.2+ and hybridized to the oligonucleotide arrays in the presence of label control targets used for array normalization and message quantitation. After washing and staining with streptavidin-phycoerythrin conjugate, hybridization patterns were visualized using an argon laser scanning confocal microscope (Affymetix, Santa Clara, Calif.) and the fluorescence intensity images processed and quantitated by GeneSeq software (Affymetrix, Santa Clara, Calif.).

Detailed Description Text (189):
Various nucleic acid sequence analysis methods can be used for detecting sequence changes. In one preferred method, high density oligonucleotide arrays are used to detect the sequence changes. One advantage of using oligonucleotide arrays is that the sequence interrogation can be performed in conjunction with gene expression monitoring in a single chip.

Detailed Description Text (194):
1. High Density Array Design and Fabrication

Detailed Description Text (196):
Approximately 65,000 unique DNA probes (10.sup.7 unique probes/50 mm.sup.2 area) were synthesized on a 1.2 cm.sup.2 glass slide. A set of4 different oligonucleotide arrays that include more than 6,500 human gene sequences derived from the GenBank (http://www.ncbi.nlm.nih.gov) and dbEST databases were generated. These arrays were used to monitor and compare the expression of>6,500 genes in parallel from normal and malignant breast tissue cell lines.

Detailed Description Text (198):
Labeled RNA for array hybridization experiments was derived from the malignant breast cell line MDA 468 and MDA231 and normal breast tissue from primary cell line HT-125. HT-125 was obtained from a cell line derived from normal breast tissue peripheral to an infiltrating ductal carcinoma. The normal and malignant cells were harvested, lysed and Poly A.sup.+ RNA isolated and used as template for double stranded cDNA (ds cDNA) synthesis using an oligo dT primer containing a T7 promoter sequence at its 5' end. ds cDNA product then served as template in an in vitro transcription (IVT) reaction using T7 polymerase and biotinylated ribonucleotides.

Detailed Description Text (200):
The labeled cRNA was then fragmented in the presence of heat and Mg.sup.2+ and hybridized to the oligonucleotide arrays in the presence of label control targets used for array normalization and message quantitation. After washing and staining with streptavidin-phycoerythrin conjugate, hybridization patterns were visualized using an argon laser scanning confocal microscope (Affymetix, Santa Clara, Calif.) and the fluorescence intensity images processed and quantitated by GeneSeq software (Affymetrix, Santa Clara, Calif.).

Detailed Description Text (201):
From Table 2, the expression of p53 activated targets gadd45, cyclin G, p21waf1, Bax, IGF-BP3 and Thrombospondin in MDA468 and MDA231 is lost. A coincident gain of expression was seen of the p53 repressed targets c-myc and PCNA These expression patterns in BT-474 indicated a loss of wild-type p53 function in both MDA468 and MDA231. A p53 genotyping array analysis confirmed heterozygous mutations in MDA438 and MDA231.

Detailed Description Text (205):
An understanding of the molecular basis of disease requires the ability to detect

genetic variation across a large number of genes and to correlate genetic factors with the resulting cellular consequences. The use of high density oligonucleotide (nucleic acid) arrays provided genotyping of candidate genes as well as the characterization of the relative abundance of mRNAs identified herein. Information from the human genome project, Merck EST sequencing effort, or any other source of genetic sequence information may be used to design and fabricate such oligonucleotide arrays for the highly parallel analysis of mRNA levels. DNA arrays containing probes that are complementary to 6,600 human ESTs were used in the particular experiments outlined herein to identify such messenger RNAs. These arrays were used to generate normal and breast cancer specific gene expression profiles. Expression levels of 137 genes increased in malignant breast cells>10-fold compared to normal breast cells. The expression of a further 167 genes decreased to near undetectable levels. A total of 1,549 expressed genes were detected in the breast cancer cells. A simple categorization of the expression changes revealed patterns characteristic of loss of wild-type p53 function, as well as increases in the Her2/neu oncogene and its signal transduction pathway, including Grb-7, Ras, Raf, Mek and ERK. Genotyping of the p53 locus using a DNA re-sequence analysis array revealed inactivating mutations in the p53 DNA binding domain and loss of heterozygosity, consistent with the functional profile given by the expression monitoring array. These data demonstrate how gene expression profiles can be used to characterize the functional state of a cell, and suggest a general array hybridization based approach to decipher specific biochemical pathways and generate new testable hypotheses.

Detailed Description Text (206):
The expressed genes identified herein will find application in a wide array of uses. Included among such uses are diagnostic uses, prognostic uses, therapeutic uses, and forensic uses.

Detailed Description Text (207):
The particular arrays designed herein utilized semiconductor based photolithography and solid phase chemical synthesis to directly synthesize independently specified DNA probes on derivitized glass at a density of 10.sup.7 oligonucleotide molecules per 50 .mu.m.sup.2 synthesis region, as discussed in Lockhart, D. J., Dong, H., Byrne, M. C., Follettie, M. T., Gallo, M. V., Chee, M. S., Mittmann, M., Wang, C., Kobayashi M., Horton, R & Brown, E. L, Expression monitoring by hybridization to high-density oligonucleotide arrays. Nature Biotechnology 13,1675-1680 (1996), incorporated herein by reference. Approximately 65,000 unique 50 .mu.m.sup.2 synthesis areas were synthesized in parallel on a 1.28 cm.sup.2 glass slide. Such high density oligonucleotide arrays applied to gene expression monitoring are specific, sensitive and quantitative, allowing detection of message levels down to one copy per cell. In the particular applications discussed herein oligonucleotide arrays were generated with probes selected from 6,600 EST gene clusters derived from the dbEST public database, as described in Boguski, M. S., Lowe, T. M. & Tolstoshev, C. M. dbEST-database for `expressed sequence tags`. Nature Genetics 4, 332-333 (1993), incorporated herein by reference. These arrays are complementary to 3,200 human full length GenBank genes, and 3,400 ESTs that demonstrate strong homology to other eukaryotic genes in the SwissProt protein sequence database.

Detailed Description Text (208):
Particular arrays herein contained collections of 20 probe pairs for each of the 6,600 messages being monitored. Each probe pair is composed of a 25-mer oligonucleotide that is perfectly complementary to a region of sequence from a specific message, and a sister probe that is identical except for a single base substitution in a central position. This combination of perfect and mismatched probes serves as an internal control for hybridization specificity and allows for sensitive quantitation in the presence of cross-hybridization backgrounds. Probes were selected on the basis of uniqueness and hybridization specificity. The aim was to choose probes that would yield the best discrimination between perfect match and single base mismatch hybridization events.

Detailed Description Text (209):
Labeled RNAs for array hybridization experiments were derived from the malignant breast cell line BT-474 and normal primary breast tissue cell line HT-125. BT-474 was isolated from a solid, invasive ductal carcinoma of the breast and is tumorigenic in athymic nude mice, as described in Lasfargues, E. Y., Coutinho, W. G. & Redfield, E. S. Isolation of two human tumor epithelial cell lines from solid breast carcinomas. Journal of the National Cancer Institute 4, 967-978 (1978), incorporated herein by reference. HT-125 was obtained from normal breast tissue peripheral to an infiltrating ductal carcinoma Hackett, A. J., Smith, H. S., Springer, E. L., Owens, R. B.,

Nelson-Rees, W. A., Riggs, J. L. & Gardner, M. B. Two syngeneic cell lines from human breast tissue: the aneuploid mammary epithelial (Hs578T) and the diploid myoepithelial (Hs578Bst) cell lines. Journal of the National Cancer Institute 6, 1795-1806 (1977). mRNA was isolated from normal and malignant cells and converted into double stranded cDNA (ds cDNA) using an oligo dT primer containing a T7 promoter sequence at its 5'-end (5). ds cDNA product (with T7 polymerase promoter sequence incorporated) served as template in an in vitro transcription (IVT) reaction using T7 polymerase and biotinylated ribonucleotides. The biotinylated cRNA was then fragmented by heating and hybridized to the oligonucleotide arrays. After washing and then staining with streptavidin-phycoerythrin conjugate, hybridization patterns were visualized using an argon ion laser scanning confocal microscope. The fluorescence intensity images were processed and quantitated by GeneChip data analysis software.

Detailed Description Text (210):
Hybridization patterns of total message from normal and malignant breast cells to sets of 20 probe pairs from 1,650 gene sequences (one array of a set of 4 encompassing 6,600 human genes) were obtained. Clear examples of unchanged and altered patterns of gene expression can be observed by visual comparison of the fluorescence intensities of probe sets from these two samples. The quantitative analysis of hybridization patterns is based on the assumption that for a specific mRNA the perfect-matched (PM) probes will hybridize more strongly on average than their mis-matched (MM) partners. The average difference in intensity between PM and MM hybridization signals is computed together with the average of the logarithm of the PM/MM ratios for each probe set. These values are then used to determine the relative copy number of a detected message.

Detailed Description Text (211):
Added biotinylated control cRNAs (E. coli biotin synthetase genes bioB, bioC, bioD and bacteriophage P1 Cre recombinase) at known concentrations served as internal standards to allow relative quantitation of fluorescence intensities for estimates of copy number per cell. Spiking experiments were performed to investigate the absolute hybridization intensity range between multiple RNAs at known concentrations. When 32 individual cRNAs were spiked at levels ranging from copy numbers of 1:100,000 to 1:30,000 in the background of total cellular mRNA, absolute hybridization intensities were within a 2-fold range for all targets tested. Added biotinylated control oligonucleotide together with endogenous cellular RNA messages (e.g. .beta.-Actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)), allowed for normalization of experimental variation and array hybridization. Intensities from the spiked standards in the presence of total cellular target demonstrated sensitivities as high as 1:100,000, corresponding to a few copies per cell. Comparison of hybridization signal intensities, that ranged over 4-orders of magnitude, revealed all categories of message expression changes including repressed (>10-fold down), down-regulated (<10-fold down), up-regulated (<10-fold up) and induced (>10-fold up) mRNAs between normal and malignant cells as shown in FIGS. 8A and 8B.

Detailed Description Text (218):
To investigate the cause of the transcriptionally inactive p53, genomic p53 was resequenced in BT474. The strategy for rapid, simultaneous analysis of large amounts of genetic information using high-density oligonucleotide arrays has been described in Chee, M., Yang, R, Hubbell, E., Berno, A., Huang, X. C., Stern, D., Winkler, J., Lockhart, D. J., Morris, M. S. & Fodor, S. P. Accessing genetic information with high-density DNA arrays, Science 5287, 610-614 (1996). The DNA array used in this study allowed for simultaneous analysis of both sense and anti-sense sequence of p53 coding exons 2-11, including 10 base pairs of intronic flanking sequence (to identify splice donor-acceptor mutations), as well as allele specific probes for over 300 characterized hotspot p53 mutations and every possible single base deletion (Dee et al., manuscript in prep.) The re-sequence analysis portion of the DNA array consisted of a set of 4 identical 20-mer oligonucleotides complementary to p53 wild-type sequence, except that an A,C,G or T was substituted into each probe at a centrally localized position. In each set of four probes, the perfect complement to the target sequence will hybridize more strongly than the single base mismatched probes (see FIG. 3A, wild-type), allowing unambiguous sequence assignment by automated basecalling software (30). By usingsets of 4 probes in this manner that span 1,490 bp of the p53 sequence, a single person can fully genotype the p53 gene from 60 genomes in the time it would take to do 12 by conventional gel-based de novo sequencing.

Detailed Description Text (219):
To facilitate characterization of mutations in the p53 gene we applied an algorithm that performs base identification of nucleotide changes between a sample and a

reference. This sequence analysis is based on two major effects that a single base change has on the array hybridization pattern of an experimental sample relative to a wild-type reference: 1) The probe containing the substitution base displays the strongest signal of the 4 probe set; and 2) The neighboring probes that overlap the position display a characteristic loss of signal or "footprint" for probes flanking a base substitution, as these probes would have a single base mismatch to the mutated target sequence distinct from the query base (see FIG. 3A, BT-474 versus wild-type, and ref 30).

Detailed Description Text (220):
The analysis of BT-474 versus HT-125 p53 genomic DNA using the p53 genotyping array revealed a single base substitution of G to A in exon 8 (DNA binding domain), resulting in an amino acid change at position 285 from E to K (FIG. 3B). The hybridization signal difference centered about the mutation identified by the footprint analysis (see FIG. 3B, top panel), and subsequent base calling of a single genotype (see FIG. 3B, bottom panel) in BT-474 by the GeneChip software indicated that this carcinoma had a loss of heterozygosity at the p53 locus (confirmed by dideoxy sequence analysis). These data unambiguously show that the loss of wild-type p53 transcriptional function in these cells was due to the absence of wild-type p53 protein. We have applied this analysis to other breast carcinomas with similar outcomes correlating altered gene expression patterns of targets of p53 transcriptional modulation with mutant p53 gene status (Table 3).

Detailed Description Text (221):
Systematic expressed genome sequencing is producing EST data at a rate that predicts ESTs to all human genes will be available in just a few years. To fully exploit the expressed sequence database, an understanding of gene function from gene sequence is required. Oligonucleotide arrays can provide a basis for genome-wide expression analysis and offer insights into regulatory interactions and gene function.

Detailed Description Text (225):
Gene expression army hybridization and scanning. 10 .mu.g of biotinylated cRNA target was fragmented to an average size of 50 nucleotides in 10 .mu.l of magnesium fragmentation buffer (40 mM Tris-acetate (pH 8.1), 100 mM KOAc, 30 mM MgOAc) at 95.degree. C. for 35 min. The fragmented samples were brought up to a final volume of 200 .mu.l with hybridization buffer (0.9 M NaCl, 60 mM NaH.sub.2 PO.sub.4, 6 mM EDTA and 0.005% Triton X-100, pH 7.6 (6.times.SSPE-T)) containing 0.1 ng/ml Herring Sperm DNA, 50 pM biotin-labeled control oligo (5'-GTCAAGATGCTACCGTTCAG-3') and biotinylated cRNA quantitation standards bioB (1.5 pM), bioC (5.0 pM), bioD (25 pM) and Cre (100 pM). Samples were denatured at 95.degree. C. for 10 min, chilled on ice for 5 min and equilibrated to room temperature (5 min) before being applied to the array flow cell. Arrays were hybridized at 40.degree. C. for 14-16 hr with rotation at 60 rpm, followed by 10 wash cycles (2 drain-fills/cycle) at room temperature with 6.times.SSPE-T in the GeneChip Fluidics Station (RELA). For staining of hybridized target cRNA, arrays were first washed in 0.5.times.SSPE-T at 40.degree. C. for 15 min with rotation (60 rpm), then incubated with 2 .mu.g/ml of phycoerytherin-strepavidin conjugate (Molecular Probes) in 6.times.SSPE-T containing 1 mg/ml of acetylated-bovine serum albumin at 40.degree. C. for 10 min. Prior to scanning, the arrays were washed at room temperature with 6.times.SSPE-T for 5 cycles (2 drains-fills/cycle) in the fluidics station. The hybridized stained arrays were scanned using an argon-ion laser GeneChip scanner 50 (Molecular Dynamics) with a resolution setting of 7.5 .mu.m/pixel (.about.45 pixels/probe cell), and wavelength detection setting of 560 nm. Fluorescence images and quantitative analysis of hybridization patterns and intensities were performed using GeneSeq Analysis Software and GEprocess (Affymetrix) gene expression data analysis programs.

Detailed Description Text (226):
p53 PCR and labeling for re-sequence analysis by array hybridization. The p53 gene was genotyped by amplifying coding exons 2-11 in a 100 .mu.l multiplex PCR reaction using 100 ng of genomic DNA extracted from cells using a QIAmp Blood Kit (Qiagen). PCR Buffer II (Perkin-Elmer) was used at 1.times. along with 2.5 mM MgCl.sub.2, 200 .mu.M of each dNTP and 10 units of Taq Polymerase Gold (Perkin-Elmer). The multiplex PCR was performed using 10 exon-specific primers (Table 4) with the following cycling conditions: 1 cycle at 94.degree. C. (5 min), 50 cycles of 94.degree. C. (30 sec), 60.degree. C. (30 sec) and 72.degree. C. (30 sec), followed by 1 cycle at 72.degree. C. (7 min). 45 .mu.l of the PCR reaction was then fragmented and dephosphorylated by incubation at 25.degree. C. for 15 min with 0.25 units of Amp Grade DNAse I (Gibco/BRL) and 2.5 units of Calf Alkaline Phosphatase (Gibco/BRL), followed by heat-inactivation at 99.degree. C. for 10 min. The fragmented PCR products were then labeled in a 100

.mu.l reaction using 10 .mu.M flourecein-N6-ddATP (Dupont-NEN) and 25 units of terminal transferase (Boehringer Mannheim) in 200 .mu.M K-Cacodylate, 25 nM Tris-HCl (pH 6.6), 0.25 mg/ml BSA and 2.5 mM CoCl.sub.2. The labeling reaction was incubated at 37.degree. C. for 45 min and heat-inactivated at 99.degree. C. for 5 min.

Detailed Description Text (227):
p53 re-sequence analysis array hybridization and scanning. The fragmented, labeled PCR reaction was hybridized to the p53 re-sequence analysis array in 6.times.SSPE-T containing 2 mg/ml BSA and 1.67 nM fluorescein-labeled control oligo (5'-CTGAACGGTAGCATCTTGAC-3') at 45.degree. C. for 30 min. The array was then washed with 3.times.SSPE-T at 35.degree. C. for 4 cycles (10 drains-fills/cycle) in the GeneChip Fluidics Station (RELA). The hybridized p53 array was scanned using an argon-ion laser scanner (Hewlett-Packard) with a resolution setting of 6.0 .mu.m/pixel (.about.70 pixels/probe cell) and wavelength detection setting of 530 nm. A fluorescence image was created, intensity information analyzed and nucleotide determination made by GeneChip Analysis Software (Affymetrix). Footprint analysis was done using Ulysses Analysis Software (Affymetrix) essentially as described.

Detailed Description Text (228):
Genotyping through-put capabilities. Conventional gel-based dideoxynucleotide sequencing can genotype approximately twelve p53 genomes a day (10 hr) assuming an average read of 400 nucleotides per gel, run twice a day. The through-put of the GeneChip p53 system for a single person, using one fluidics station and scanner (40 min hyb/wash and 6 min scan time) is approximately 6 arrays per hour, or sixty p53 genomes fully genotyped in a 10 hour period.

Detailed Description Text (229):
Gene expression array oligonucleotide probe selection and array desgin. The probes for the human 6,600 gene arrays were selected from the 600 bases at the 3'-end of sequences chosen from the dbEST database. Probes for inclusion on the arrays were identified based on a criteria of uniqueness and hybridization characteristics. Uniqueness was accessed by comparing potential probes with all genes that were considered for inclusion on the arrays. If any potential probe matched 22 out of 25 nucleotides of another sequence that probe was discarded. Selection of probes for hybridization characteristics was done by using heuristic rules and a neural net developed from previous expression experiments. The heuristics for the 6,600 gene arrays were as follows: 1) total number of As or Ts less than 13; 2) total number of Cs or Gs less than 11; 3) number of As or Ts in a window of 8 less than 7; 4) number of Cs or Gs in a window of 8 less than 6; 5) palindrome score less than 9 (the palindrome score is a measure of probe self-complementarity). The neural net was used to prune out probes that it identified as poor hybridizers or promiscuous cross hybridizers as described in detail elsewhere. Finally, any probes requiring more than 70 synthesis steps to include on the arrays were rejected to minimize synthesis time and cost.

Detailed Description Text (230):
Data from tables 2 & 3 include expression results from an array designed to identify alternatively spliced forms of targets. This array surveys 250 genes from functional categories including oncogene, tumor suppressor, DNA mismatched repair and apoptosis gene products. Probe pairs for this design were chosen such that each exon for a given message was represented on the array. In this way, specific loss of signal from a sub-set of probes corresponding to a particular exon of a message would indicate a splice variant form.

Detailed Description Text (233):
The present invention provides greatly improved methods, compositions, and apparatus for identifying gene function and for studying the regulatory relationship among genes. It is to be understood that the above description is intended to be illustrative and not restrictive. Many variations of the invention will be apparent to those of skill in the art upon reviewing the above description. By way of example, the invention has been described primarily with reference to the use of a high density oligonucleotide array, but it will be readily recognized by those of skill in the art that other nucleic acid arrays, other methods of measuring transcript levels and gene expression monitoring at the protein level could be used. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled.

Other Reference Publication (4):
Lipshutz et al. Using Oligonucleotide Probe Arrays to Access Genetic Diversity,

BioTechniques, Sep. 1995, vol. 19, No. 3, pp. 442-447.

Other Reference Publication (6):
Schena et al. Parallel human genome analysis: Microarray-based expression monitoring of 10000 genes. Proc. Natl. Acad Sci. USA Oct. 1996, vol. 93, No. 20, pp. 10614-10619.

Other Reference Publication (7):
Chee et al. Accessing Genetic Information with High-Density DNA Arrays, Science, Oct. 5, 1996, vol. 274, No. 5287, pp. 610-614.

Other Reference Publication (11):
March Cheeet al. "Accessing Genetic Information with High-Density DNA Arrays" Science, ISSN 0036-8075 Elsevier Science, 1996, vol. 5287, pp. 610-614.

Other Reference Publication (31):
March Schena et al. "Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray" Science, ISSN 0036-8075, Elsevier Science, 1995, pp. 467-470.

Other Reference Publication (33):
David J. Lockhart "Expression monitory by hybridization to high-density oligonucleotide arrays" Nature biotechnology, 1996, pp. 1675-1680.

Other Reference Publication (41):
Chee et al. Accessing Genetic Information with High-Denisty DNA Arrays. Science. Oct. 5, 1996, vol. 274, No. 5287, pp. 610-614, see entire document.

CLAIMS:

5. The method of claim 4, wherein said amount of transcripts is determined with a high density nucleic acid array.

NCBI formatting BLAST

Nucleotide Protein Translations Retrieve results for an RID

Your request has been successfully submitted and put into the Blast Queue.

Query = (731 letters)

Your search was limited by an Entrez query: Homo sapiens [ORGN]

The request ID is 1043365164-010551-8443

Format! or Reset all

The results are estimated to be ready in 4 seconds but may be done sooner.

Please press "FORMAT!" when you wish to check your results. You may change the formatting options for your result via the form below and press "FORMAT!" again. You may also request results of a different search by entering any other valid request ID to see other recent jobs.

Format

Show ☑ Graphical Overview ☑ Linkout ☑ Sequence Retrieval ☑ NCBI-gi Alignment in HTML

Number of: Descriptions 100 Alignments 50

Alignment view Pairwise

Format for PSI-BLAST ☐ with inclusion threshold: 0.005

Limit results by entrez query or select from: (none)

Expect value range: